The Correlation between Hepatitis C Core Antigen and Hepatitis C Virus RNA Levels with Respect to Human Immunodeficiency Virus Status, Hepatitis C Virus Genotype and Interferon-Lambda-4 Polymorphism

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Key Words

Hepatitis C core antigen · Viral load · Hepatitis C genotype · HIV coinfection · Interferon-lambda-4 polymorphism

Abstract

Objectives: Serum hepatitis C virus (HCV) core antigen (HCVcAg) concentrations correlate with HCV RNA levels in HCV monoinfected patients. Data in HCV/HIV coinfecting patients are still limited. We aim to compare the use of HCVcAg measurement with respect to HIV status, HCV genotypes, interferon-lambda-4 (IFNL4) polymorphism and clinical parameters. **Methods:** We analyzed an untreated cohort of 104 patients with HCV mono-infection and 85 patients with HCV/HIV coinfection. Serum HCVcAg was measured by a commercial chemiluminescent microparticle immunoassay. The presence of IFNL4 polymorphism ss469415590 was identified by real-time PCR. **Results:** log₁₀ HCVcAg levels were significantly correlated with corresponding log₁₀ HCV RNA levels ($r = 0.889$, $p < 0.001$), but not with ALT levels and liver stiffness. The correlation between HCV RNA and HCVcAg was particularly high in coinfecting patients and those with high viremia. Mean log₁₀ HCVcAg concentration was signifi-

cantly higher in coinfecting patients than in mono-infected patients. Patients harboring the TT/TT genotype of ss469415590 had significantly higher levels of log₁₀ HCVcAg than those with the non-TT/TT genotype. HCVcAg levels were similar across HCV genotypes. **Conclusions:** HCVcAg concentrations had an excellent correlation with HCV RNA levels, particularly in HCV/HIV-coinfecting individuals and might be associated with IFNL4 polymorphism. HCVcAg testing could be used as an alternative to HCV RNA assays in resource-limited settings.

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Introduction

Hepatitis C virus (HCV) infection is a major cause of liver fibrosis, cirrhosis and hepatocellular carcinoma, affecting up to 170 million people worldwide [1]. HCV has been classified into seven major genotypes, all of which display different patterns of geographic distribution. In Thailand, approximately 2.2% of the general population is chronically infected with HCV, and the most common genotypes are genotype 3 (HCV-3), gen-

otype 1 (HCV-1) and genotype 6 (HCV-6) [2]. The screening tests for HCV infection in clinical practice rely on the detection of anti-HCV antibodies using enzyme-linked immunosorbent assays (ELISA). However, these tests cannot differentiate between resolved HCV infection and an active viral replication. Therefore, the measurement of serum HCV RNA generally serves to confirm the diagnosis of an ongoing infection [3]. In addition, monitoring of HCV RNA is crucial for assessing the treatment response to antiviral therapy [4]. Currently, real-time polymerase chain reaction (PCR)-based quantitative determination is the diagnostic gold standard. However, this assay is limited in developing countries due to costs and the requirement for a real-time PCR machine.

Recently, a highly sensitive assay for measuring serum HCV core antigen (HCVcAg) concentrations using a fully automated chemiluminescent microparticle immunoassay has become commercially available [5]. A number of studies have demonstrated good correlation between HCVcAg and HCV RNA levels, particularly in HCV-monoinfected individuals [6–13]. As a result, HCVcAg testing is considered to be an alternative assay to conventional HCV RNA quantification with the advantages of rapidity, reproducibility and feasibility in settings with limited resources [14]. To date, data regarding the correlation between HCVcAg and HCV RNA levels in patients coinfecting with HIV are still limited [15–17]. In addition, the effect of different viral genotypes, particularly HCV-6 prevalent in Southeast Asia [18], on HCVcAg levels and the correlation with HCV RNA levels remains to be established.

Genome-wide association studies have reported an association between single nucleotide polymorphisms (SNPs) adjacent to the interleukin-28B (*IL-28B*) gene (principally rs12979860) and treatment response in patients with chronic HCV infection [19–21]. Recently, a transiently induced region (interferon-lambda-4; *IFNL4*) harboring a dinucleotide variant ss469415590 (TT or ΔG) showed strong linkage disequilibrium to rs12979860 [22]. So far, this novel ss469415590 SNP is the only functional variant identified in association with HCV clearance [23] and also a good predictor of treatment response to pegylated IFN and ribavirin therapy [24]. However, it is unclear whether there is an association between this SNP and HCVcAg concentrations in patients with chronic HCV infection. Thus, the aims of this study were to evaluate the use of HCVcAg measurement with respect to HIV status, HCV genotypes, ss469415590 polymorphism and clinical parameters.

Materials and Methods

Patients

HCV/HIV coinfecting patients, who were seropositive for both anti-HCV and HCV RNA, were enrolled consecutively from the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT, Bangkok, Thailand). To compare the diagnostic role of HCVcAg between coinfecting and mono-infected individuals, serum samples were obtained from patients with chronic HCV monoinfection, who were positive for both anti-HCV antibody and HCV RNA. These mono-infected patients were selected randomly from a pool of patients with chronic liver disease who were followed-up at King Chulalongkorn Memorial Hospital (Bangkok, Thailand). None of the patients enrolled in this study had hepatitis B virus (HBV) infection or received any antiviral therapy for chronic HCV infection when the blood sample was obtained. Written informed consent and ethical approval by the institutional review board was obtained for all patients. All blood samples were stored at –80°C until examined.

HCV RNA Quantification and Genotypes

HCV RNA quantification was performed using real-time quantitative reverse transcription PCR (Abbott Molecular Inc., Des Plaines, Ill., USA) in accordance with the manufacturer's instructions. The lower and upper detection limits of the assay were <12 IU/ml and 100,000,000 IU/ml, respectively. HCV genotypes were determined by nucleotide sequencing of the core and NS5B regions followed by phylogenetic analysis as described previously [2].

HCVcAg Quantification

Quantification of HCVcAg was performed using a fully automated CMIA according to the manufacturer's instructions (Abbott Diagnostics, Tokyo, Japan). The assay allows the quantitative determination of HCVcAg in a linear range from 3 to 20,000 fmol/l, with the possibility of 1:9 automated dilutions that extend the assay linearity to 180,000 fmol/l [5].

IFNL4 Genotyping

The presence of *IFNL4* polymorphism ss469415590 was identified by reverse transcription PCR assay using TaqMan genotyping assay with MGB probes (Applied Biosystems, USA) as described previously [25]. Briefly, genomic DNA of patients with chronic HCV infection was extracted from 100 µl of peripheral blood mononuclear cells using the QIAamp DNA Mini Kit (Qiagen, Germany). The reaction mixture consisted of 1 µl of DNA extract, 200 nM of each probe (ss469415590_IFNL4_VIC: 5'-ATCGCAGAAGGCC-3' and ss469415590_IFNL4_FAM: 5'-ATCGCAGCGGCC-3'), 900 nM of each primer (ss469415590_IFNL4_F: 5'-GCCTGCTGCAGAAGCAGAGAT-3' and ss469415590_IFNL4_R: 5'-GCTCCAGCGAGCGGTAGTG-3') (Applied Biosystems), 5 µl of 2× Perfect Taq Plus Master Mix (5 PRIME, Gaithersburg, Md., USA) adjusted to 10 µl of final volume by distilled water. The PCR conditions were 2 min at 50°C, 10 min at 95°C, 45 cycles of 15 s at 95°C and 2 min at 60°C. The fluorescent signal was detected at the end of each cycle. The genotype of ss469415590 was analyzed by applying the Endpoint Genotyping method (Light Cycler 480; Roche Diagnostics, USA). For this SNP, TT and ΔG are defined as major and minor alleles, respectively.

Liver Stiffness Measurement

After fasting for at least 2 h, liver stiffness measurement was obtained from each patient using transient elastography (FibroScan; Echosens, Paris, France) according to the manufacturer's instructions. Results were recorded in kilopascals (kPa) as the median value of all measurements. The procedure was based on at least 10 validated measurements: the success rate (ratio between numbers of validated and total measurements) was over 60% and the interquartile range was less than 30% [26].

Data Analysis

A Mann-Whitney U test or Student's test was used to compare continuous variables, and a χ^2 test or Fisher's exact test was used to compare categorical variables. The Spearman rank correlation was used to evaluate the relationships between variables. All data were analyzed by using SPSS Statistic Software Package for Windows version 20.0 (SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

A total of 189 patients with chronic HCV infection were included in this study. There were 104 patients with HCV mono-infection and 85 patients with HCV/HIV coinfection. Table 1 summarizes the demographic and clinical characteristics of the patients. There were no significant differences in the baseline characteristics between each group in terms of mean age, BMI, mean ALT level, and the distribution of HCV genotypes and ss469415590 genotypes. There was a higher proportion of females among the HCV mono-infected patients in comparison to the HCV/HIV coinfecting patients ($p < 0.001$). In contrast, HCV/HIV coinfecting patients had significantly higher mean liver stiffness, $\log_{10}$ HCV RNA and $\log_{10}$ HCVcAg levels compared with HCV mono-infected patients ($p = 0.004$, 0.018 and 0.035 , respectively).

Correlation between $\log_{10}$ HCVcAg and $\log_{10}$ HCV RNA Levels

In this study, serum HCV RNA and HCVcAg levels were detected in all cases. Mean $\log_{10}$ HCV RNA was 6.1 ± 1.0 IU/ml (range: 3.4–7.9 IU/ml) and mean $\log_{10}$ HCVcAg was 3.6 ± 0.6 fmol/l (range: 1.9–4.6 fmol/l). In the overall cohort, $\log_{10}$ HCVcAg levels significantly correlated with corresponding $\log_{10}$ HCV RNA levels ($r = 0.889$, $p < 0.001$) as shown in figure 1. Regarding HIV status, there were significant correlations between both markers in mono-infected patients ($r = 0.845$, $p < 0.001$) and in coinfecting patients ($r = 0.939$, $p < 0.001$). However, the mean $\log_{10}$ HCV RNA/HCVcAg ratio was not sig-

Fig. 1. Correlation between $\log_{10}$ HCVcAg and $\log_{10}$ HCV RNA levels in all patients.

Table 1. Demographics and characteristics of HCV-mono- and -coinfecting patients

Characteristics	Mono-infection (n = 104)	Coinfection (n = 85)	p
Age, years	45.6±9.7	44.1±7.2	n.s.
Sex			
Males	61 (58.7)	74 (87.1)	
Females	43 (41.3)	11 (12.9)	<0.001
BMI, kg/m ²	23.9±4.2	22.6±6.5	n.s.
ALT, U/l	74.3±55.6	88.8±53.1	n.s.
Liver stiffness, kPa	9.1±5.6	14.1±12.0	0.004
CD4+ T cell counts, cell/μl	441.4±216.6		
HCV RNA, $\log_{10}$ IU/ml	6.0±1.0	6.3±1.0	0.018
HCVcAg, $\log_{10}$ fmol/l	3.5±0.6	3.7±0.5	0.035
HCV genotypes			
1	38 (36.5)	29 (34.1)	
3	41 (39.4)	44 (51.8)	
6	25 (24.1)	12 (14.1)	n.s.
IFNL4 genotypes			
TT/TT	92 (88.5)	74 (87.1)	
TT/ΔG	7 (6.7)	10 (11.8)	
ΔG/ΔG	5 (4.8)	1 (1.1)	n.s.

Data are presented as means ± SD or n (%). ALT = Alanine aminotransferase.

nificantly different between mono-infected and coinfecting patients (1.69 ± 0.18 vs. 1.70 ± 0.12 , respectively, $p = 0.774$).

Additionally, we investigated the correlation between both markers in terms of HCV viremia. Overall, high viral

Table 2. Serum levels of log₁₀ HCV RNA and log₁₀ HCVcAg according to HCV genotypes

	Genotype 1 (n = 67)	Genotype 3 (n = 85)	Genotype 6 (n = 37)	p
HCV RNA, log ₁₀ IU/ml	6.0±0.9	6.1±1.0	6.4 ± 1.1	0.227
HCVcAg, log ₁₀ fmol/l	3.5±0.5	3.6±0.6	3.8 ± 0.6	0.058

load samples (log₁₀ HCV RNA ≥5.0 IU/ml) showed a better correlation ($r = 0.815$, $p < 0.001$) than low viral load samples (log₁₀ HCV RNA <5.0 IU/ml ($r = 0.555$, $p < 0.001$)). The same trends were also observed in the monoinfected group ($r = 0.731$, $p < 0.001$ and $r = 0.462$, $p = 0.015$, respectively) and the coinfecting group ($r = 0.902$, $p < 0.001$ and $r = 0.724$, $p = 0.003$, respectively). Regardless of HIV status, mean log₁₀ HCV RNA/HCVcAg ratio was significantly higher in the high viral load group compared with the low viral load group (1.72 ± 0.13 vs. 1.63 ± 0.23 , respectively, $p = 0.002$).

Correlation between log₁₀ HCVcAg Levels and HCV Genotypes

The correlation between log₁₀ HCVcAg and log₁₀ HCV RNA levels was also evaluated with respect to HCV genotypes. Our data showed that log₁₀ HCVcAg levels significantly correlated with log₁₀ HCV RNA across HCV genotypes with the best correlation found in HCV-3 [HCV-1 ($r = 0.796$, $p < 0.001$), HCV-3 ($r = 0.919$, $p < 0.001$) and HCV-6 ($r = 0.895$, $p < 0.001$)]. When comparing the levels of log₁₀ HCV RNA and log₁₀ HCVcAg among different HCV genotypes, we did not observe any significant differences although patients with HCV-6 had slightly higher levels of both markers than the other groups (table 2). In addition, the levels of both markers regarding HIV status were not significantly different across HCV genotypes (data not shown).

The relationship between HCV genotypes and clinical parameters was further examined. Patients with HCV-3 infection had significantly higher mean ALT levels than patients with HCV-6 infection (90.8 ± 53.6 vs. 60.0 ± 50.2 U/L, respectively, $p = 0.004$), but did not significantly differ from patients with HCV-1 infection (79.8 ± 56.1 U/L, $p = 0.229$). There was no significant difference in liver stiffness among patients infected with HCV-1, HCV-3 and HCV-6.

Table 3. Serum levels of log₁₀ HCV RNA and log₁₀ HCVcAg according to ss469415590 genotypes

	TT/TT genotype (n = 166)	Non-TT/TT genotype (n = 23)	p
HCV RNA, log ₁₀ IU/ml	6.2±1.0	5.6±1.0	0.014
HCVcAg, log ₁₀ fmol/l	3.6±0.6	3.3±0.5	0.004

Correlation between log₁₀ HCVcAg Levels and ss469415590 Genotypes

Since a relatively small proportion of individuals displayed TT/ΔG or ΔG/ΔG genotypes of ss469415590, we grouped these genotypes together for statistical analysis. As shown in table 3, patients harboring a TT/TT genotype had significantly higher levels of log₁₀ HCV RNA than those with a non-TT/TT genotype (6.2 ± 1.0 vs. 5.6 ± 1.0 IU/ml, respectively, $p = 0.014$). A similar observation was found for log₁₀ HCVcAg levels (3.6 ± 0.6 vs. 3.3 ± 0.5 fmol/l, respectively, $p = 0.004$).

In the monoinfected group, there was no significant difference between patients with TT/TT and non-TT/TT genotypes in terms of log₁₀ HCV RNA (6.0 ± 0.9 vs. 5.4 ± 1.2 U/L, respectively, $p = 0.119$) and log₁₀ HCVcAg levels (3.6 ± 0.6 vs. 3.3 ± 0.6 fmol/l, respectively, $p = 0.193$), although a trend towards increases in both markers were observed among the TT/TT genotype. In the coinfecting group, patients harboring the TT/TT genotype had significantly higher levels of log₁₀ HCV RNA and log₁₀ HCVcAg than those with the non-TT/TT genotype (6.4 ± 1.0 vs. 5.8 ± 0.8 U/L, respectively, $p = 0.034$, and 3.8 ± 0.5 vs. 3.3 ± 0.3 fmol/l, respectively, $p = 0.002$).

The relationship between the ss469415590 genotypes and clinical parameters was also explored. Regarding baseline serum ALT level, there was no significant difference between patients with TT/TT and non-TT/TT genotypes (83.8 ± 56.1 vs. 60.7 ± 40.2 U/L, respectively, $p = 0.058$), although a trend towards increased ALT levels was observed among the TT/TT genotype group. Similarly, there was no significant difference in liver stiffness between patients with TT/TT and non-TT/TT genotypes (11.9 ± 10.1 vs. 13.9 ± 11.2 kPa, respectively, $p = 0.543$).

Correlation between HCVcAg Levels and Clinical Parameters

In addition to associations with HCV genotypes and ss469415590 genotypes, the relationship between

HCVcAg levels and clinical parameters was examined. In this study, $\log_{10}$ HCVcAg levels were not correlated with serum ALT levels ($r = -0.045$, $p = 0.542$) and liver stiffness ($r = -0.122$, $p = 0.155$). Similarly, $\log_{10}$ HCV RNA levels were not correlated with serum ALT ($r = -0.031$, $p = 0.676$) and liver stiffness ($r = -0.078$, $p = 0.365$). We also analyzed the correlation between HCVcAg levels and CD4+ T cell counts in the coinfection group. In this study, $\log_{10}$ HCVcAg levels were not correlated with CD4+ T cell counts ($r = 0.093$, $p = 0.396$). Similarly, $\log_{10}$ HCV RNA levels were not correlated with CD4+ T cell counts ($r = 0.063$, $p = 0.569$).

Discussion

Detection of HCVcAg levels in the serum has emerged as a potential marker for active HCV infection and may be used to evaluate response to antiviral therapy and disease progression. Several recent data have demonstrated that serum HCVcAg concentrations correlate well with HCV RNA levels in HCV monoinfected patients [6–12]. In the present study, we further investigated the use of HCVcAg measurement in HCV/HIV coinfecting patients. In particular, we directly compared the use of HCVcAg measurement between HCV monoinfected and HCV/HIV coinfecting individuals, with respect to HCV genotypes, host genetic variations and other disease parameters that could influence the clinical outcome of chronic HCV infection.

Previous studies estimated that the sensitivity of the ARCHITECT HCVcAg assay is approximately 3 fmol/l, which corresponds to 500–2,500 IU/ml of HCV RNA [7, 8, 12]. Thus, the concordance of detectable HCV RNA and HCVcAg of all samples in this cohort likely resulted from high viremia, which is a typical feature of untreated individuals. Our data confirmed previous reports that HCVcAg levels had a good correlation with the corresponding HCV RNA levels in both the mono- and coinfection groups. It should be noted that the correlation between both assays appeared to be higher among the coinfecting group than the monoinfected group ($r = 0.939$ and $r = 0.845$, respectively). Indeed, the correlation coefficients between the two markers in coinfecting patients exceeded 0.90 in most previous studies [15–17]. For example, a recent cross-sectional survey conducted in China demonstrated that the correlation coefficients between HCVcAg and HCV RNA concentrations was higher in coinfecting than monoinfected subjects ($r = 0.952$ and $r = 0.808$, respectively) [17]. Another study conducted in Germany showed that the correlation coefficients be-

tween these two markers in HCV/HIV coinfection was 0.97 compared to 0.75 in HCV monoinfection [16].

It should be mentioned that the correlation between HCVcAg and HCV RNA levels was also typically high among other groups of patients with impaired immune response, including liver and kidney transplant recipients [27] and patients with end-stage kidney disease [28]. In contrast, such correlation was less consistent among immunocompetent individuals. Thus, it appears that the correlation between both markers of HCV replication might be in part influenced by the immune status of infected individuals. The explanation of these interesting findings are unclear, but might be related to an interaction between HCVcAg and anti-HCV antibodies. In general, it was proposed that anti-HCV antibodies might mask the detection of HCVcAg or could lead to an enhanced clearance of circulating antigen. However, among patients with impaired immune response including HIV-infected individuals or patients receiving immunosuppressive therapy, lower antibody levels presumably do not interfere with the measurement of HCVcAg [16, 27]. In contrast, patients with HBV/HCV coinfection did not show good correlation between the two markers [16], which might result from the complexity of viral interaction and fluctuation of virological dominance over time [29]. Collectively, these data indicate that HCVcAg measurement is an excellent surrogate marker for monitoring HCV viral replication in HCV/HIV coinfecting patients.

Of note, mean serum concentrations of HCV RNA and HCVcAg in our study were significantly higher in coinfecting than monoinfected groups. These results are consistent with previous reports that HCV/HIV coinfection is associated with persistent HCV viremia and higher HCV viral load [30]. The increased HCV RNA levels among coinfecting patients are thought to be in part associated with the decline in CD4+ and CD8+ T cell responses to HCV infection. Our data also showed that coinfecting patients had significantly higher liver stiffness, representing more advanced liver fibrosis compared to monoinfected patients. These findings are in agreement with previous studies that the prevalence of significant liver fibrosis is usually high among coinfecting individuals [31]. In the context of HCV/HIV coinfection, emerging data have suggested that multiple conditions, including increases in profibrogenic cytokine expression and secretion, enhancement of oxidative stress production, and increases in hepatocyte apoptosis all contribute to accelerated fibrosis [32]. In contrast to previous studies, our results did not demonstrate the correlation between HCVcAg concentration and liver inflammatory activity and liver fibrosis [33].

Unlike previous reports of HCVcAg, our study included a proportion of patients infected with HCV-6. As mentioned previously, the data on this HCV genotype are sparse due to its restricted distribution in South China and Southeast Asia [18]. In this study, we observed slightly different correlations between HCVcAg and HCV RNA levels across HCV genotypes with the best correlations found in HCV-3, followed by HCV-6 and HCV-1, respectively. However, HCVcAg and HCV RNA levels did not vary according to HCV genotypes. Of note, samples with high viral load showed a better correlation of HCVcAg and HCV RNA concentrations than those with low viral load. In addition, the mean HCV RNA/HCVcAg ratio was significantly higher among samples with high viremia compared to those with low viremia. These observations were presumably related to a higher proportion of unmasked HCVcAg detectable by the HCVcAg assay in the setting of high viremia, as described previously in HCV/HIV coinfecting patients and transplant recipients [16, 27].

Recent reports have shown that the ΔG variant of ss469415590 is associated with poorer HCV clearance and response to antiviral therapy than the TT allele [22, 24]. However, the association of this polymorphism with HCVcAg levels remains to be elucidated. To our knowledge, our study is the first to assess IFNL4 genetic status in relation to HCVcAg levels in patients with chronic HCV infection. We found that patients harboring the TT/TT genotype had significantly higher levels of HCV RNA and HCVcAg than those with the non-TT/TT genotype, regardless of HIV status and HCV genotypes. This finding is consistent with previous data in that the favorable CC genotype of rs12979860, which is associated with bet-

ter treatment response, and is also related to higher baseline viral load [19, 34]. The effects on HCV RNA and HCVcAg concentrations might be in part related to biological interaction between HCV replication and IFNL4 protein. As shown in previous studies, the IFNL4 ΔG variant could induce weak expression of IFN-stimulated genes, which in turn provides an antiviral response in reducing HCV viral load. In contrast, the ΔG variant also diminishes the responsiveness to type I and type III IFN required for effective treatment during antiviral therapy [22, 35]. More information is needed to understand the mechanisms that underlie this association, as well as the clinical impact of ss469415590 polymorphisms on HCVcAg detection.

In conclusion, our data showed that there was an excellent correlation between HCV RNA and HCVcAg concentrations, particularly in HCV/HIV coinfecting individuals. We also demonstrated that serum levels of HCVcAg were associated with ss469415590 polymorphism. As the HCVcAg assay is a reliable test and has the advantages of being rapid and reproducible, its measurement could be used as an alternative to HCV RNA assays in resource-limited settings.

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